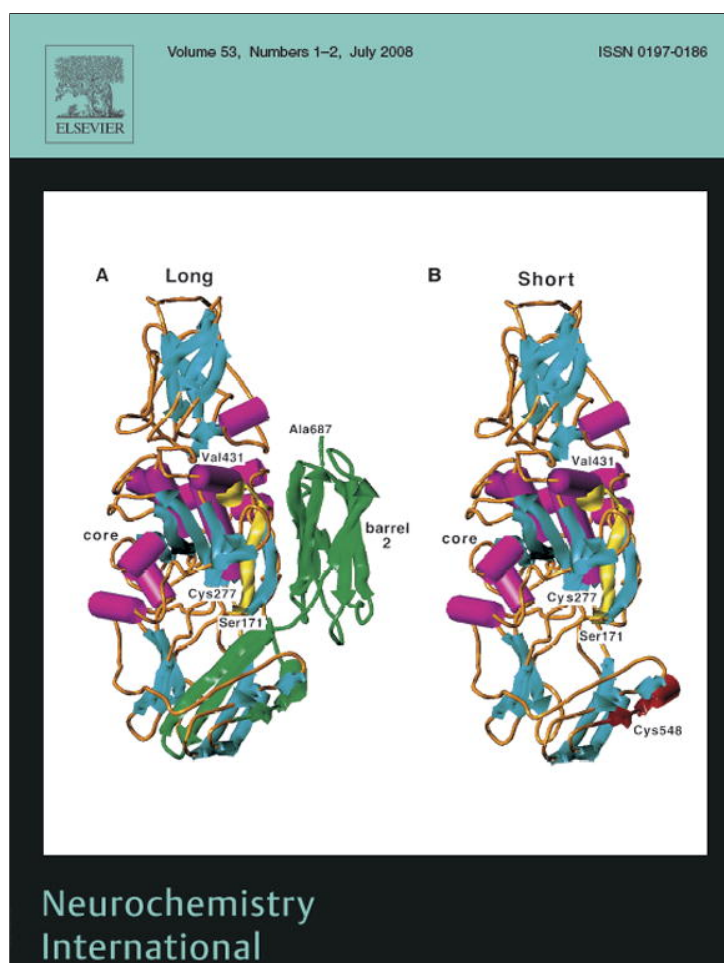


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journal homepage: www.elsevier.com/locate/neuintDespite irreversible binding, PET tracer [^{11}C]-SA5845 is suitable for imaging of drug competition at sigma receptors—The cases of ketamine and haloperidolRudie Kortekaas^{a,b,*}, R. Paul Maguire^b, Aren van Waarde^c, Klaus L. Leenders^b, Philip H. Elsinga^c^a Department of Anatomy and Embryology, University Medical Center Groningen, University of Groningen, The Netherlands^b Department of Neurology, University Medical Center Groningen, University of Groningen, The Netherlands^c Department of Nuclear Medicine and Molecular Imaging, University Medical Center Groningen, University of Groningen, The Netherlands

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ABSTRACT

Many psychotropic compounds bind to sigma receptors and several new sigma ligands are in development for psychiatric indications such as anxiety, attention deficit hyperactivity disorder, depression and psychosis. Of special interest for drug development are tomographic methods that can quantify the binding of promising sigma ligands in a regional manner. Here we present the development of such a method and the first evaluation of sigma ligand [^{11}C]-SA5845 in a primate. Extensive pharmacokinetic modeling was done on tissue curves and a heart lumen curve. The effects of pretreatment and challenge with haloperidol were studied as well as those of pretreatment with ($\pm$)-ketamine. The tracer had a plasma half-life of 77 ± 1.7 min and was rapidly taken up by all brain areas. The binding pattern was consistent with binding to sigma receptors and compartment modeling showed there was considerable specific binding that was irreversible. We therefore calculated the net influx rate, K_i , with the Gjedde–Patlak linearization, as a measure of free receptors. As expected, K_i was very sensitive to the presence of competing ligands (–)-ketamine and/or haloperidol. Summarizing, the tracer is well suited for visualizing sigma receptors in the brain and moreover, the presented method is able to quantify, on a regional basis, specific binding of unlabeled ligands to sigma receptors.

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Sigma (σ) receptors are densely expressed in the brain and two subtypes have been identified: σ_1 and σ_2 . Currently a number of brain penetrating σ ligands are in development as potential new central nervous system (CNS) drugs for psychiatric, addiction and neurological treatment.

Since σ_1 agonists are hallucinogenic, it is conceivable that antagonists are antipsychotics. Tardive dyskinesia, a side effect that may occur during prolonged treatment with haloperidol may be σ_2 mediated. It was hypothesized that σ_2 agonists haloperidol and its reduced metabolite induce neuronal death via this receptor leading to tardive dyskinesia (Bowen, 2000). If this is the case, σ_2 antagonists could be helpful in limiting or preventing these motor side effects (Bowen, 2000).

Cocaine, methamphetamine and phencyclidine are substances with addictive potential and all are σ receptor ligands (Sharkey et al., 1988; Sharp, 1997; Nguyen et al., 2005). The alleged anti-

addictive compound ibogaine (Alper et al., 1999; Mash et al., 2000) binds with high affinity (90–250 nM) to the σ_2 subtype (Mach et al., 1995), and this has been proposed as mediating its anti-addictive properties (Helsley et al., 1998).

P-glycoprotein is a neuroprotective molecular pump that appears to be hypofunctional in the midbrain of Parkinson's disease patients (Kortekaas et al., 2005). Because σ_2 agonists reduce the expression level of P-glycoprotein (Bowen, 2000), antagonists could be useful in neuroprotection. If σ_2 antagonists increase P-gp expression in the blood–brain barrier, reduced entry of neurotoxins may retard disease progression. This effect, if verified, could be further enhanced by a presumed anti-apoptotic effect of σ_2 antagonists (Crawford and Bowen, 2002).

The development of new CNS drugs can be greatly accelerated by the use of neuroimaging methods that are able to quantify the binding of drugs to neuroreceptors. PET and also single photon emission tomography (SPET) are very suitable techniques to this end, provided that a tracer with the right characteristics is used. The tracer should be metabolically stable and its pharmacokinetics should be understood. It should have a rapid brain uptake with high specific binding to the receptor population of interest. Binding of test compounds should be quantifiable with an intuitive and

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stable (i.e., reproducible) hyperparameter. Ideally a data analysis method would permit quantification of competition on a regional level.

[¹¹C]-SA5845 (“compound **1b**”) is a metabolically stable compound with a high *in vitro* affinity for both σ_1 and σ_2 receptors: 33 and 9.5 nM (IC₅₀) respectively (Kawamura et al., 2003). It has high uptake in the mouse and rat brain and *ex vivo* autoradiography of the rat brain indicates binding to σ receptors (Kawamura et al., 2003).

This is the first evaluation of this compound in a primate model. In addition to the visualization of σ receptors, our aim was to develop a method to quantify competition from unlabeled test ligands. To this end we used a previously developed method to measure the plasma curve with the PET scanner (Kortekaas et al., 2004) and did extensive pharmacokinetic modeling.

1. Experimental procedures

1.1. Subject

This study was approved by the institution's animal ethics committee and complied with national laws on care and use of laboratory animals.

An adult (13 years old) male rhesus monkey of 8 kg was scanned four times. There were at least 21 days between any two consecutive experiments. The monkey was fasted from the night previous to the experiment.

During handling and transportation it was sedated with intramuscular ketamine, which also functioned as anesthetic induction. As specified per experiment below, ketamine was either the racemic mixture ($\pm$)-ketamine (Nimatek, Eurovet, Bladel, The Netherlands) or (+)-ketamine (Ketanest-S, Pfizer, Hoofddorp, The Netherlands). Subsequently a cannula was inserted into the saphenous vein for administration of drugs and radiotracer. Intramuscular atropine (0.06–0.09 mg/kg; Centrafarm, Etten-Leur, The Netherlands) and sodium pentobarbital (8–11 mg/kg intravenous; Sanofi, Maassluis, The Netherlands) were administered, after which the trachea was intubated for ventilation with 1–2% isoflurane in medical oxygen (Abbott, Kent, UK).

The animal was positioned in a styrofoam primate chair which was built for the purpose of having brain and heart inside the field of view in order to obtain a cardiac input curve without the need for arterial sampling (Kortekaas et al., 2004). Heart rate, electrocardiogram, respiration rate and oxygen saturation were monitored continuously.

1.2. Radiochemistry, drug administration and PET scans

A transmission scan was performed for attenuation correction with the internal rotating ⁶⁸Ge/⁶⁸Ga rod sources of the PET scanner (ECAT HR+, Siemens, Erlangen, Germany).

The tracer 1-([4-methoxy-¹¹C]-3,4-dimethoxyphenethyl)-4-[3-(4-fluorophenyl)-propyl]piperazine ([¹¹C]-SA5845) was synthesized in 40 min by methylation of the 4-O-demethyl precursor with [¹¹C]-methyl iodide (Kawamura et al., 2003). The decay-corrected radiochemical yield was 4–12%, specific radioactivity was 12–14 TBq/mmol and radiochemical purity was >95%.

A total of 100–270 (median 169) MBq of activity was dissolved in 5 ml of sterile saline containing 10% ethanol, and administered as a fast intravenous bolus. The acquisition of the PET scanner was started simultaneously. PET data were reconstructed to a 128 × 128 × 63 matrix with a plane separation of 0.2425 cm and a bin size of 0.2250 cm.

Because both haloperidol and (–)-ketamine bind to σ receptors, four different experiments were performed—*block* experiment: anesthesia with ($\pm$)-ketamine, block with haloperidol at $t = -30$ min; *early challenge 1*: anesthesia with ($\pm$)-ketamine, challenge with haloperidol at $t = +60$ min; *early challenge 2*: anesthesia with (+)-ketamine, challenge with haloperidol at $t = +60$ min; *late challenge*: anesthesia with (+)-ketamine, challenge with haloperidol at $t = +120$ min. Haloperidol dose was always 1 mg/kg, intravenous, times are relative to tracer injection.

Acquisition was in 24 frames totaling 90 min for *block*, in 42 frames totaling 120 min for *early challenge* and in 48 frames totaling 180 min for *late challenge*. Frames were 10 up to 600 s depending on expected kinetics.

1.3. Metabolites

Venous blood samples were taken at 1, 5, 10, 20, 40, 60, 65, 70 and 80 min after the start of the scan and analyzed with a previously described thin layer chromatography analysis (Elsinga et al., 2004). After development, the chromatography plate was cut into narrow strips (width ca. 3 mm) and radioactivity in each strip was measured with a gamma counter (Compugamma CS1282, LKB-Wallac, Turku, Finland). Afterwards, the fraction of plasma radioactivity corresponding to intact [¹¹C]-SA5845 was calculated as $100\% \times (\text{counts attributable to } [^{11}\text{C}]\text{-SA5845}) / (\text{counts attributable to } [^{11}\text{C}]\text{-SA5845} + \text{counts attributable to metabolites})$.

This is plotted against time in Fig. 2. In the *block* experiment the curve could not be constructed beyond $t = 20$ due to technical problems.

1.4. ROI analysis

Region of interest (ROI) analysis was used to generate time–activity curves (TACs). For visualization, the activity concentration was normalized to the activity concentration at 30 min, this in order to account for variations in injected dose. TACs also functioned as input for the Patlak analysis (Gjedde, 1982; Patlak et al., 1983) and the compartment models. Patlak analysis is essentially a two-parameter fit whereas the compartment models were four- and five-parameter fits. For this reason larger ROIs, with higher signal to noise ratios, were used for the compartment models.

Using the application AMIDE (Loening and Gambhir, 2003), ellipsoid ROIs were defined in the PET images and TACs were constructed by plotting normalized activity concentration against time.

1.5. Patlak linearization

Since the binding of this tracer was irreversible (see Section 2), we employed Patlak analyses on ROI TACs (Gjedde, 1982; Patlak et al., 1983) with metabolism-corrected heart TAC as input function. If a haloperidol injection was made during the scan, time points after the injection were excluded from the fit. For each ROI, the transformed data were inspected visually to identify the start point of the linear part of the curve. The resulting hyperparameters are *net influx rate* (K_i) and *distribution volume of the non-specific compartment* (V_f). K_i the steady-state uptake rate constant (in min^{-1}) of the tracer and equals $(K_1 k_3) / (k_2 + k_3)$ as indicated in Fig. 1b (Gjedde, 1982; Patlak et al., 1983). V_f represents “the vascular plus steady-state space of the reversible tissue region” (Gjedde, 1982; Patlak et al., 1983).

Since k_2 was large, changes in flow had little effect on K_i , which could thus be used as index of specific binding. In case the metabolite data were unavailable for the entire scan, the curve was extrapolated with a monoexponential function through ($t = 0$, $y = 100\%$).

1.6. Compartment modeling

Data were fitted to both models using the Simplex Algorithm (Nelder and Mead, 1965).

1.6.1. Whole body model

The metabolite and parent ratios and the TACs for the heart were used as input for a five-parameter whole body model (Fig. 1a). The model allowed for labeled ligand in blood as well as tissue and for labeled metabolites, which were assumed to remain in the blood pool. This was based on chromatographic analysis where one metabolite was found which was highly polar. The proportions of metabolites were fixed to chromatography findings.

1.6.2. Tissue model

TACs for brain and cerebellum were used as input for a four-parameter, two-tissue (plus plasma) compartment model (Fig. 1b). Only these large ROIs were used in order to increase precision. Hyperparameters K_i , V_f and *distribution volume of the bound compartment* (V_b) were calculated from these. V_b equals $V_f \times k_3 / k_4$ as indicated in Fig. 1b.

1.7. Parametric images

Standardized uptake value (SUV) images were calculated as the integral over the period 30–60 min of (activity concentration in Bq/ml)/(administered dose in Bq/weight of monkey in gram). Using a voxel-wise Patlak analysis (as above), parametric images encoding K_i and V_f were made. The appropriate start time for the Patlak fit was the one determined with the ROI data. All parametric images were smoothed by spatial trilinear interpolation.

2. Results

In our thin layer chromatography system, the parent compound [¹¹C]-SA5845 had an average R_f value of 0.7 (range 0.5–1.0), while

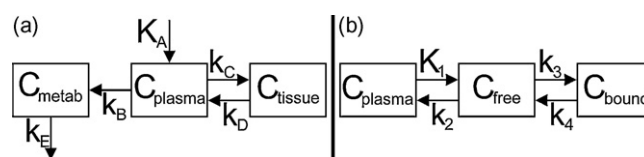


Fig. 1. Compartment models and nomenclature of rate constants. (a) Whole body model for all radioactive entities and (b) tissue model for [¹¹C]-SA5845.

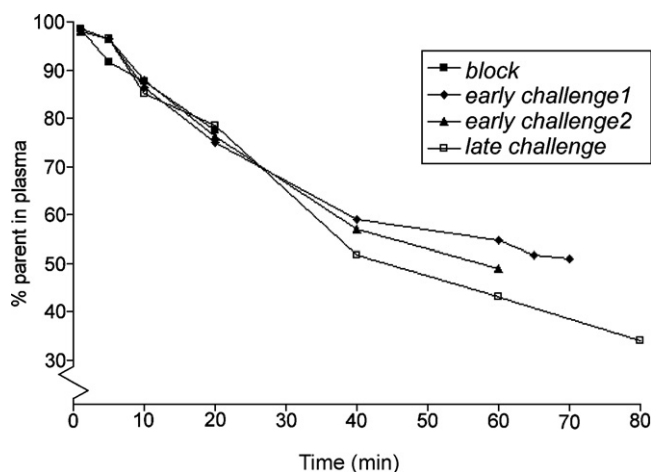


Fig. 2. Metabolic conversion of the tracer [^{11}C]-SA5845 in four experiments as established with thin layer chromatography. Experiment codes are indicated, see Section 1.2.

one polar metabolite appeared around $R_f = 0.0$. The chemical identity of this metabolite was not investigated. The $T_{1/2}$ for this conversion was estimated directly from the graphs in Fig. 2 by exponential regression, thus ignoring distribution of [^{11}C]-SA5845. This resulted in an average ($\pm$ standard deviation) $T_{1/2}$ of 56 (± 7.6) min. See below for an estimate of $T_{1/2}$ based on compartment modeling which accounted for brain uptake and hence presumably is more accurate.

For the different experiments the parameter estimates for the whole body model (Fig. 1a) are given in Table 1. [^{11}C]-SA5845 was metabolized (k_B) with an average ($\pm$ standard deviation) half-life of 77 (± 1.7) min.

Parameter and hyperparameter estimates for the tissue model (Fig. 1b) and Patlak analysis are in Table 2. All parameters could be estimated reliably except k_4 (k_{off}) and hence also hyperparameter V_b representing the distribution volume of the 'bound compartment'. The rate constants K_1 and k_2 were large, while k_4 was, as stated, very small.

Uptake of [^{11}C]-SA5845 was evaluated with SUV parametric images. Peripheral areas with high SUV were: thyroid gland, sternum, heads of humeri, vertebrae, myocardium, liver and spleen (Fig. 3). Brain areas with a high SUV (Fig. 5) were cingulate gyrus, septal nuclei, striatum, insula, thalamus, midbrain and cerebellum (esp. anteroventral parts of vermis and intermediate hemisphere).

Brain uptake was investigated with a ROI analysis. Brain TACs for all experiments reached a plateau ($\pm 5\%$) within 30 min (Fig. 4). In the case of haloperidol pretreatment, the curve started to decline

Table 1

Parameter estimates for whole body model (Fig. 1a)

Experiment	K_A	k_B (min^{-1})	k_C (min^{-1})	k_D (min^{-1})	k_E (min^{-1})
Block	290	0.0090	1.80	0.34	0.19
Early challenge 1	249	0.0094	0.93	0.37	0.10
Early challenge 2	310	0.0090	1.30	0.40	0.14
Late challenge	200	0.0089	2.30	0.23	0.25

Parameter k_B represents the formation of a radioactive metabolite from [^{11}C]-SA5845.

again after 30 min. In the other cases a plateau developed that remained almost constant until a haloperidol challenge was given. The challenge at 60 min caused a steeper decrease in the TAC than the challenge at 120 min.

Haloperidol caused a large decrease ($\approx 30\%$) in SUV in the entire brain while the presence of (–)-ketamine reduced frontal SUV more than cerebellar SUV. Like in the ROI-based counterpart, the parametric K_i image was very sensitive to the pretreatment, with $\approx 30\%$ decreases after ($\pm$)-ketamine and $\approx 70\%$ decreases after ($\pm$)-ketamine and haloperidol. V_f was slightly decreased after ($\pm$)-ketamine and slightly increased after ($\pm$)-ketamine plus haloperidol.

A Patlak analysis was performed on TACs for a range of ROIs (Fig. 6). The fit was restricted to the linear portion of the curve. In all cases the curve was linear for time points ≥ 40 min.

In general, K_i was lower in peripheral areas than in the brain. Brain K_i was very sensitive to pretreatment. Highest K_i values were found after (+)-ketamine, much lower values after ($\pm$)-ketamine and even lower values after ($\pm$)-ketamine plus haloperidol. In vertebrae and lung there was a similar ordering of K_i values relative to pretreatment. V_f was high in liver and spleen, low in thyroid and bone. Brain regions had intermediate V_f values that displayed no obvious dependency on pretreatment.

3. Discussion

This is the first evaluation of the tracer [^{11}C]-SA5845 for imaging of σ receptors in a primate. By virtue of extensive pharmacokinetic modeling we were able to conclude specific, irreversible binding to σ receptors, that was nevertheless very sensitive to pharmacological competition.

3.1. Brain uptake

Uptake in the brain was high (SUVs of 3–5) and relatively fast: TACs reached a plateau ($\pm 5\%$) within 30 min (Fig. 4). High uptake of [^{11}C]-SA5845 occurred in the cingulate gyrus, septum, striatum, insula, thalamus, midbrain and cerebellum (Fig. 5), in accordance with the literature on σ receptor ligands (Elsinga et al., 2002, 2004).

Table 2

Parameter and hyperparameter (K_i , V_f , V_b) estimates for tissue model (Fig. 1b) and Patlak method

Experiment	Region	Tissue model								Patlak method	
		Delay	K_1 (min^{-1})	k_2 (min^{-1})	k_3 (min^{-1})	k_4 (min^{-1})	K_i (min^{-1})	V_f	V_b^a	K_i (min^{-1})	V_f
Block	Brain	0.000	0.161	0.117	0.017	0.009	0.020	1.37	2.49	0.015	1.951
	Cerebellum	0.000	0.188	0.122	0.015	0.011	0.021	1.54	2.18	0.014	2.113
Early challenge 1	Brain	0.072	0.318	0.275	0.036	0.005	0.037	1.16	9.00	0.030	1.241
	Cerebellum	0.117	0.456	0.287	0.031	0.005	0.044	1.59	9.08	0.033	1.687
Early challenge 2	Brain	0.000	0.352	0.211	0.037	0.002	0.052	1.67	33.54	0.051	1.419
	Cerebellum	0.000	0.453	0.215	0.035	0.002	0.063	2.11	37.15	0.055	1.687
Late challenge	Brain	0.045	0.219	0.106	0.020	0.000	0.035	2.07	5.44E+7	0.058	1.926
	Cerebellum	0.000	0.292	0.118	0.023	0.003	0.048	2.48	22.31	0.068	2.447

Hyperparameter estimates derived from both methods were similar in magnitude: correlation of $r = 0.759$ for K_i and $r = 0.618$ for V_f .

^a V_b estimates are unreliable due to very small k_4 (i.e., irreversible binding).

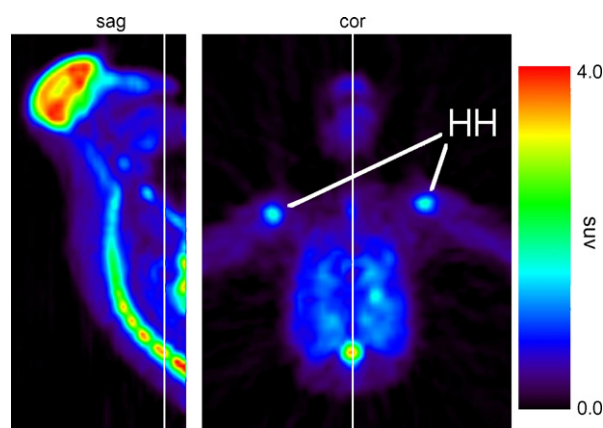


Fig. 3. Standardized uptake value images of [^{11}C]-SA5845 in the head and upper trunk of a rhesus monkey (anesthesia with (+)-ketamine, no haloperidol). Activity concentration was averaged over $t = 30$ –60 min. In each panel, the white line indicates the level of section in the other panel. sag: sagittal section, cor: coronal section and HH: heads of humeri.

3.2. Metabolism

With a five-parameter, three-compartment model (Fig. 1a) the metabolic half-life was calculated at ≈ 77 min. The major metabolite was a highly polar species as indicated by zero migration speed on silica gel. This makes it unlikely that this metabolite contributed to the radioactivity signal from the brain. Both these aspects of [^{11}C]-SA5845 metabolism are considered favorable for a PET tracer.

3.3. Specific binding

The markedly altered kinetics after haloperidol pretreatment (*block* experiment; Fig. 4) indicate that specific binding was considerable. The difference between the *block* curve and the pre-challenge parts of the *challenge* curves can be used to estimate specific binding (not shown). Specific binding rose slowly from zero at $t = 0$ to about half the total brain content of [^{11}C]-SA5845 at 90 min. SUV parametric images (Fig. 5) show that the presence of (–)-ketamine esp. in combination with haloperidol interfered with uptake of [^{11}C]-SA5845 in the brain. Patlak analysis on both ROI and voxel data (Figs. 6 and 5) further confirmed high specific binding in the brain since K_i was markedly reduced by the presence of (–)-ketamine and further by the presence of haloperidol in addition to (–)-ketamine. K_i was highest after (+)-ketamine, $\approx 30\%$ decreased after ($\pm$)-ketamine and $\approx 70\%$ decreased after ($\pm$)-ketamine and haloperidol. A similar orderly reduction in K_i occurred in liver, vertebrae and lung, suggesting that specific binding to σ sites

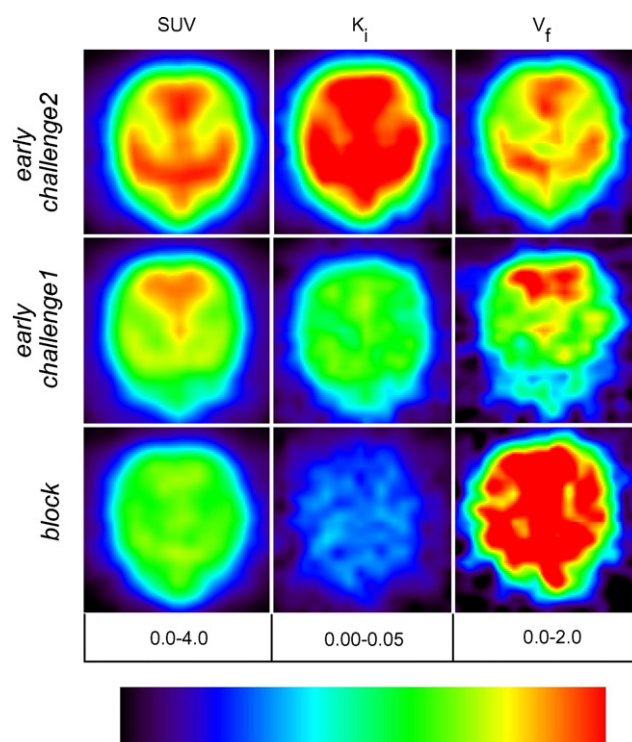


Fig. 5. Parametric images of the uptake of [^{11}C]-SA5845 in the head of a rhesus monkey for three experiments. A transaxial plane at the level of the thalamus is shown with the nose pointing downwards. SUV: standardized uptake value, the activity concentration was averaged over $t = 30$ –60 min, K_i is a measure of specific binding, V_f for non-specific binding. Experiment codes are indicated, see Section 1.2. Calibration is at the bottom of each column. (Only results of one of the two experiments with (+)-ketamine are shown, the others were similar but more noisy.)

also occurred in these organs. The σ receptors in vertebrae and the heads of the humeri are likely associated with red bone marrow, because this is a highly proliferative tissue (Mach et al., 1997). These results confirm that (–)-ketamine and haloperidol bind to σ sites (Bowen et al., 1988; Øye et al., 1991) and that competition occurs between tracer and haloperidol (Elsinga et al., 2002, 2004).

3.4. Binding was irreversible

In the absence of haloperidol the tracer concentration in the brain rose to plateau without ever decreasing (Fig. 4), which can be an indication of entrapment in the brain. The long plateau was abolished by pretreatment with haloperidol which suggests that entrapment was due to σ receptor binding in the brain. Patlak analysis on all ROI TACs produced straight lines, which is a positive

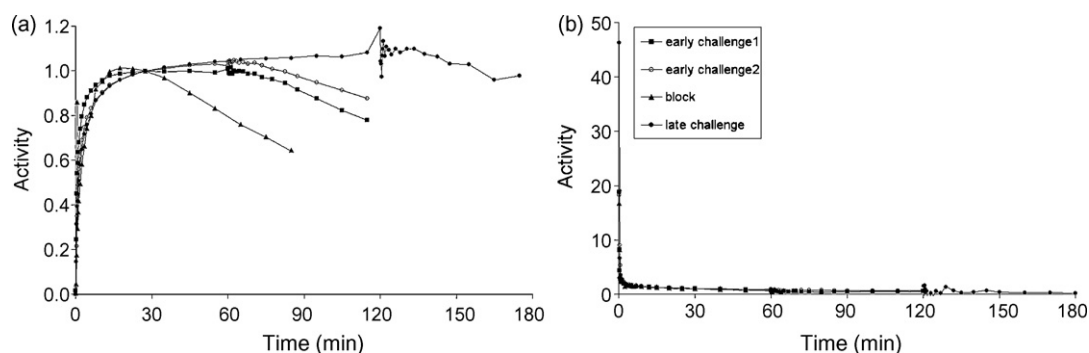


Fig. 4. Time-activity curves for (a) the whole brain and (b) the heart in the four experiments. Activity concentration in arbitrary units. Experiment codes are indicated, see Section 1.2.

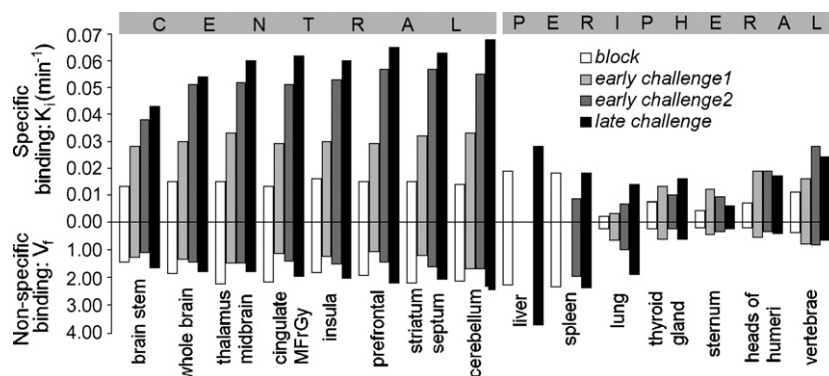


Fig. 6. Specific and non-specific binding as established by the Patlak analysis on time–activity curves for a selection of regions of interest. Experiment codes are indicated, see Section 1.2. MFGy: medial frontal gyrus and prefrontal: prefrontal cortex. (Liver and spleen were sometimes outside the field of view.)

confirmation of irreversible binding. Compartment modeling further confirmed that efflux from the specific compartment (k_4 or k_{off}) was very small for this tracer so that specific binding can be considered irreversible on this timescale. A previous study with this tracer in mice also found constantly rising brain concentrations over 1 h suggestive of irreversible binding (Kawamura et al., 2003).

3.5. Apparent tracer displacement by haloperidol challenge

Haloperidol challenge caused a sudden decrease in activity concentration in all ROIs in the brain (only shown for whole brain—Fig. 4), especially when administered at 60 but not so much at 120 min. This may appear incompatible with the conclusion that [¹¹C]-SA5845 bound irreversibly to σ receptors. However, when total binding is separated into specific and non-specific binding, either by subtracting the haloperidol + (–)-ketamine curve or by compartment modeling, a superior explanation becomes evident. The non-specific binding component washed in quickly and then washed out from $t = 20$ min. The specific binding component was slow and irreversible. During the plateau phase of the TAC the washout from the non-specific compartment approximately canceled the entrapment in the specific compartment. When association of [¹¹C]-SA5845 to σ receptors was suddenly blocked by haloperidol, the net uptake in the brain became negative revealing the washout of the non-specific component. This is illustrated by the fact that the slopes of the apparent displacement phases were the same as the slope of the haloperidol + (–)-ketamine curve at the corresponding time point (Fig. 4) indicating that the presence of haloperidol only blocked association of tracer to the receptor while leaving already bound tracer untouched.

3.6. Comparison of [¹¹C]-SA5845 with similar tracers

Metabolic stability of [¹¹C]-SA5845, indicated by plasma half-life, appeared better (≈ 56 min) than that of [¹¹C]-SA4503 (≈ 30 min), [¹⁸F]-FE-SA4503 (≈ 38 min) and [¹⁸F]-FE-SA5845 (≈ 14 min) (Elsinga et al., 2004). The formation of just one hydrophilic metabolite simplifies correction for metabolism. Speed of brain uptake of all four analogues was similar but [¹¹C]-SA5845 was roughly twice as fast as [¹¹C]-SA4503 (Ishiwata et al., 2001; Elsinga et al., 2004). Kinetics of brain uptake of [¹¹C]-SA5845 were similar to those of [¹¹C]-SA4503 and [¹⁸F]-FE-SA5845: rapid brain uptake with no washout (Elsinga et al., 2004). This is a warning that these two analogues might also bind irreversibly. Brain distribution of [¹¹C]-SA5845 was qualitatively and quantitatively similar to that of all three analogues mentioned (Elsinga et al., 2004).

3.7. Animal use

To minimize the number of animals used, this feasibility study was carried out in one animal. A practical advantage is that interindividual variability is avoided. Indeed, the reproducibility between the experiments on different days was considerable (Figs. 6 and 4). To estimate test–retest variability we used data from early challenge2 and late challenge. These experiments were performed many weeks apart and conveniently, the first 60 min of these experiments are replications in the sense that only (+)-ketamine was present. Fig. 6 shows that the hyperparameter estimates based on these two experiments (dark grey and black bars) are within a relatively close average margin (which was in fact 16%).

3.8. Ketamine anesthesia

Hyperparameter K_i , the net influx rate of tracer, is proportional to the concentration of unoccupied receptors (Wong et al., 1986). All approaches taken in this paper indicated reductions in brain K_i when ($\pm$)-ketamine was present relative to when (+)-ketamine was present. Hence, ($\pm$)-ketamine caused more occupation of σ receptors than (+)-ketamine. The difference between ($\pm$)-ketamine and (+)-ketamine is the fact that the former consists of 50% (–)-ketamine and 50% (+)-ketamine, while the latter is 100% pure (+)-ketamine. The presence of (–)-ketamine therefore is what reduced the number of unoccupied σ receptors in other words (–)-ketamine occupies σ receptors, as was known (Øye et al., 1991). Anesthesia by ($\pm$)-ketamine is very similar to (+)-ketamine anesthesia, provided that ($\pm$)-ketamine is dosed twice as high as (+)-ketamine, in other words: provided that the amount of (+)-ketamine administered is the same. This indicates that (–)-ketamine is dispensable for anesthesia and in combination with the, by now well established, σ binding of (–)-ketamine this makes (–)-ketamine a substance to be avoided during σ receptor binding studies. In other words, we recommend (+)-ketamine anesthesia over ($\pm$)-ketamine anesthesia for studies of the σ receptor.

3.9. Summary

In summary, [¹¹C]-SA5845 is a good tracer to image σ receptors in the brain with high uptake, high specific binding and good metabolic stability. Its binding to σ receptors is irreversible so that specific binding may be analyzed with the linearization according to Gjedde and Patlak (Gjedde, 1982; Patlak et al., 1983). Although this means that binding potential is very difficult to measure, the large k_2 combined with small k_4 make K_i insensitive to flow and thus a good index of specific binding (k_3 , equaling $B_{max}k_{on}$). We

demonstrated the feasibility and sensitivity of this method to quantify specific binding to sigma receptors in competition studies. This approach is both suitable and valuable for the evaluation of therapeutically promising σ receptor ligands.

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